

of TM1A helix would not protrude into the membrane core. To further characterize the conformation of the TM1A helix, we used SMD simulations and pulled this helix relative to the scaffold or the core domain. The free energy profile was also endorsed our findings in the position of TM1A. These study indicates that changes in the environment can affect the equilibrium conformation of LeuT.

2323-Pos Board B460

Computational and Experimental Investigation of the Human and D. Melanogaster Dopamine Transporters

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The existence of a solved structure for a dopamine transporter drastically changes the landscape for investigation of the mechanism of monoamine transporter function. While previous structures for LeuT have shed light on transport mechanisms, and though LeuT shares considerable structural overlap with dDAT, LeuT has no corresponding electrophysiology. The new structure therefore has the advantage of correlating structure and function to help unravel the outstanding mystery of transporters as channels, which has well developed correlates in the CIC class of Cl⁻ channels but no equivalent in monoamine transporters.

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Visualization of Molecular Events from Dopamine-Binding to -Release by Human Dopamine Transporter

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Dopamine transporter (DAT) controls neurotransmitter dopamine homeostasis by pumping excess extracellular (EC) dopamine into presynaptic neurons to prevent neurotoxicity. The process is assisted by co-transport of two sodium and one chloride ions. Malfunction of DAT has been implicated in many diseases, such as Parkinson's disease and attention deficit hyperactivity disorder. Significant advances have been made in the characterization of the structure and dynamics of a prokaryotic orthologue, LeuT, although the molecular mechanism of dopamine translocation through human DAT (hDAT) remains elusive. Recently, the first eukaryotic DAT structure was resolved from *Drosophila melanogaster*, in the outward-facing open (Ofo) state. Using homology modeling and unbiased molecular dynamics simulations, we elucidated the sequence of molecular events involved in dopamine translocation by hDAT, including the uptake from the EC medium, closure of the EC gates upon dopamine binding, passage over a holo-occluded intermediate temporarily stabilized via concerted changes in the relative orientations of the TM helices, subsequent opening of the intracellular vestibule, release of co-transported ions, and succeeding discharge of dopamine upon weakening the electrostatic attractions by aspartates near the binding-site. The transport events revealed by these hDAT simulations show striking consistency with those disclosed in recent investigation of LeuT transport cycle (1, 2). The study sheds light for the first time at the mechanism of DAT functioning at the atomic level and provides insights into methods for modulating its interactions with drugs and other substrate proteins.

1. Cheng, M.H. and I. Bahar. 2014. Complete mapping of substrate translocation highlights the role of LeuT N-terminal segment in regulating transport cycle, *PLoS Comput Biol.* in press.

2. Cheng, M.H. and I. Bahar. 2013. Coupled global and local changes direct substrate translocation by neurotransmitter-sodium symporter ortholog LeuT, *Biophys J.* 105:630-9.

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Characterization of a Secondary Site of Drug Action on the Human Dopamine Transporter

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In recent work, we employed a series of methylenedioxypyrovalerone (MDPV) analogs to determine the structural determinants for the potent nature of MDPV to inhibit uptake by the human dopamine transporter (hDAT). In hDAT-expressing *Xenopus laevis* oocytes clamped to -60 mV, MDPV and its analogs induced comparable outward currents (attributed to a block of the endogenous hDAT inward leak) that did not return to the original baseline after washout. We recorded DA-induced hDAT currents before and after application of either MDPV or its analogs, and obtained individual amplitude recovery profiles relative to the initial DA-induced currents, which correlated with the compounds' potency to inhibit DA uptake via hDAT. Interestingly, for all compounds, after

washout of the second DA application, the hDAT-mediated shift in baseline returned to the elevated level. Moreover, for two MDPV analogs the second DA response recovered 100% of the first DA response even if the baseline had shifted. Furthermore, the shift in baseline produced by one of these analogs was not impeded by the presence of a high concentration of dopamine. These results suggest two distinct sites of action for drugs targeting DAT. We employed electrophysiology to characterize this secondary site of action, and homology models of hDAT based on the crystal structure of a bacterial leucine transporter (LeuT) combined with docking simulations to identify the secondary binding site of MDPV and MDPV analogs.

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Carboxyl Residues Required for Transport by a Vesicular Monoamine Transporter Homolog from *Brevibacillus Brevis* (BbMAT)

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The uptake and storage of neurotransmitters in synaptic vesicles is carried out by neurotransmitter transporters from, e.g., the vesicular monoamine transporter (VMAT) family, which allow regulated release of monoamines from the presynaptic neuron into the synaptic cleft. Phylogenetic analysis reveals that VMAT proteins are evolutionarily related to bacterial multidrug transporters involved in antibiotic resistance. In fact, VMATs are responsible for sequestering toxic substrates such as MPP⁺ into vesicles, away from their primary site of action. To understand the molecular mechanisms of transport by VMAT, we characterized a homologous transporter from *Brevibacillus brevis* (BbMAT) using molecular modeling and biochemical approaches. First, we built a homology model of BbMAT based on the crystal structure of YajR, a putative proton-driven MFS transporter from *Escherichia coli*. As expected, the model contains 12 transmembrane (TM) helices, arranged in two domains of 6 TMs each, which are related by 2-fold pseudo-symmetry around an axis perpendicular to the membrane between the two halves. The model predicts that four carboxyl residues, a histidine, and an arginine are located in the TM segments that line the central cavity. The activity of BbMAT coupled to a proton gradient was assessed biochemically, demonstrating that the transport process is electrogenic and therefore that at least two protons are exchanged for each substrate. The six residues exposed to the aqueous cavity according to the model were then mutated either alone or in combination, and these mutants were tested for their ability to confer resistance to ethidium and acriflavine. The results show that only two residues, D25 (TM1) and E229 (TM7), are essential for proton-coupled transport in BbMAT. We conclude that BbMAT is a paradigm for the study of multidrug and neurotransmitter transport by VMATs.

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Monoamine Transporters Produce Calcium Signals through L-Type Calcium Channel Activation

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Monoamine transporters take up neurotransmitters, but they also release neurotransmitters when challenged by abused drugs like amphetamine (AMPH) or MDMA (ecstasy). Transported substrates including transmitters and drugs are accompanied by excess positive charges that constitute an inward, depolarizing current. Previous research suggests that dopamine transporter (DAT) substrate-induced currents produce bursts of action potentials in dopaminergic neurons. Both pace-making and action potential bursting are mediated by L-type Ca²⁺ channels (CaV1.3) in several excitable cells, including some dopaminergic neurons, whereas N-type Ca²⁺ channels (CaV2.2) are mainly involved in neurotransmitter release. Here we test the hypothesis that monoamine transporters are electrically coupled to L-type Ca²⁺ channels. We express the serotonin transporter (SERT) in wt and CaV1.1-null myoblasts. After differentiation, wt myotubes, but not CaV1.1-null myotubes, release Ca²⁺ after 5HT or S(+)-MDMA exposure, suggesting the participation of L-type Ca²⁺ channels. This effect was sensitive to fluoxetine but refractory to TTX, implicating SERT and excluding the participation of voltage-gated Na⁺ channels. Furthermore, co-expression of SERT & CaV1.3 in HEK cells supported Ca²⁺ signals, whereas SERT & CaV2.2 was unresponsive to 5HT or S(+)-MDMA. Similarly, co-expression of DAT & CaV1.3 or DAT & CaV1.2 supported Ca²⁺ signals induced by S(+)-AMPH or dopamine, whereas DAT & CaV2.2 expression was refractory to both agents even though S(+)